



**Human Angiogenesis ELISA Strip I (Chemiluminescence)**  
Catalog Number EA-1611 (For Research Use Only)

**Introduction**

Angiogenesis shifted from the avascular to vascular states is a key event for sustained tumor growth and cancer progression. Angiogenesis as a biological switch process is governed by numerous pro- and anti-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGFb), epidermal growth factor (EGF), and transforming growth factor-beta (TGFb). The mechanism of action of each of these factors is different, as are their origin and the stimuli for their production. The angiogenic switch refers to the balance between pro- and anti-angiogenic factors. Therefore, profiling of these factors is critical to understanding angiogenesis. Signosis' Angiogenesis ELISA Strip Profiling Assay simultaneously profiles 8 angiogenesis cytokines; TNF $\alpha$ , IGF-1, VEGF, IL-6, FGFb, TGFb, EGF, and Leptin. The difference of these proteins between two samples can be determined through data comparison.

**Principle of the assay**

In each well of the strip, a primary antibody against a specific cytokine is coated and each of the 8 wells of the strip is coated different antibodies. Therefore, each strip can measure of 8 different proteins. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. The plate is further detected with HRP luminescent substrate. Luminescence is reported as relative light units (RLUs) on a microplate luminometer. The level of expression is directly proportional to the luminescent intensity.

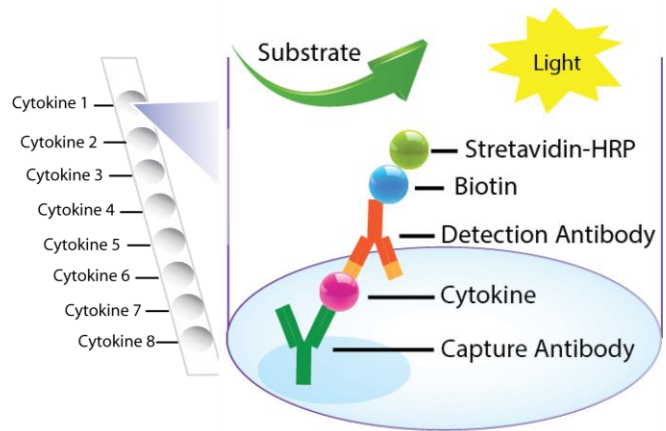


Diagram of Human ELISA Strip I (Chemiluminescence)

**Materials provided with the kit**

Component	Qty	Store at
<b>96-Well white 12 strip Plate coated with 8 different antibodies against Human Angiogenesis I cytokines</b>	1	4°C
<b>Biotin-labeled antibody mixture against 8 different Human Angiogenesis I cytokines</b>	200 $\mu$ L	-20°C
<b>Streptavidin-HRP conjugate</b>	50 $\mu$ L	4°C
<b>1xDiluent buffer</b>	40mL	4°C
<b>5X Assay wash buffer</b>	40mL	4°C
<b>Substrate A and B</b>	1mLeach	4°C
<b>Substrate dilution buffer</b>	8mL	4°C

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH<sub>2</sub>O
- Refer to Standards User Manual for diluting standards.
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

## Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer, for example, add 80ul sample in 720ul 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

## Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the cytokines among the samples can be easily identified and determined.
- If you would like to quantitatively measure the proteins in the samples, please order the corresponding standards, which can be used for making standard curves through a series of 2-fold dilutions.

## Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
  2. Standard curve:
    - If protein standard curve is desired, 4-5 strips may be used to make Standard curve.
  3. Sample assay:
    - Apply each sample in one strip, 100ul per well and incubate for 1-2 hour at room temperature with gentle shaking.
  4. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
  5. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
  6. Repeat the aspiration/wash as in step 4.
  7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
  8. Freshly prepare the substrate solution.
    - For whole plate:
      - 1 ml Substrate A
      - 1 ml Substrate B
      - 8 ml Substrate dilution buffer
  9. Invert the plate over an appropriate container and expel the contents forcibly, then firmly tap the plate against clean paper towels. Wash the plate by adding 200 ul of 1x Assay wash buffer. Incubate wash buffer for 10 minutes on a shaker. Repeat washing process two times for a total of three washes with 10 minutes incubation between each wash.
- Note: It is important to incubate wash buffer for 10 minutes during each wash to reduce background.**
10. Add 95 µl substrate solution to each well and incubate for 2 minutes.
  11. Place the plate in the luminometer. Set integration time to 1 second with no filter position and read **immediately**.

## Human Angiogenesis ELISA Strip I (Chemiluminescence) Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$
B	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1
C	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF
D	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
E	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb
F	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$
G	EGF	EGF	EGF	EGF	EGF	EGF	EGF	EGF	EGF	EGF	EGF	EGF
H	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin